DEVELOPMENTAL STAGES OF THE BROAD BREASTED BRONZE TURKEY EMBRYO [1,2]

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In studying early mortality in the turkey embryo, it became necessary to determine with considerable accuracy the extent of development of the embryo.

Phillips and Williams (1944) described the Black and the Beltsville Small White turkey embryos after different durations of incubation. However, chronological age, *i.e.*, incubation time, *per se*, is not a reliable expression of the extent of morphological differentiation of the embryo. Such factors as temperature and humidity during incubation, genetic composition, and size of the egg, have been shown to affect the rate of growth of the avian embryo (for review, see Landauer, 1951). It has previously been shown in this laboratory (Kosin and St. Pierre, 1956) that storage of Broad Breasted Bronze hatching eggs for 8 to 14 days results in a lowered mean somite count after 60 hours of incubation, as compared with eggs held for 1 to 7 days.

Hamburger and Hamilton (1951) established a series of normal stages of development for the chick embryo, based on various morphological criteria. These criteria were found to be useful in this laboratory in estimating the extent of development of the turkey embryo, although the turkey embryo takes approximately 28 days to hatch as compared with 21 days in the chicken. Thus, the major objective of the study reported in this paper was to determine for turkey embryos the period of incubation necessary to obtain the different stages of embryonic development described for the chicken by Hamburger and Hamilton (1951).

Similar studies on "staging" of embryonic development in Aves have previously been reported by Rempel and Eastlick (1957) and Koecke (1958) for the White Silkie bantam chicken and the Khaki Campbell and White Indian Runner ducks, respectively.

MATERIALS AND METHODS

All embryos used in this study were obtained from eggs produced by a flock of Broad Breasted Bronze (BBB) turkeys maintained at the Station. The birds

were trapnested and the eggs were collected three times a day, after which they were placed in the holding room at 50° F. and 85% relative humidity for not more than three to four days. The eggs were incubated for a desired length of time in a forced draft incubator at 99.5° F. The earlier embryos (1 to 7 days) were removed from the yolk, placed in chick Ringer's solution and then measured and staged. Each embryo was staged separately according to (1) the development of the mesodermal derivatives, e.g., somites; (2) the development of the ectodermal derivatives, e.g., optic vesicles, neuromeres; and (3) the development of the heart. The "average" stage of development of the embryo was then obtained from these three separate stagings. Although there were individual differences, no striking and consistent differences between the turkey and the chicken were observed in terms of rate of development in these three groups of morphological criteria. Older embryos (17 to 28 days) were fixed in Bouin's fluid or Baker's calcium formol before staging.

In the later stages of development (Hamburger-Hamilton, stage 36 to stage 40), the turkey embryo has a distinct structure, the "snood" or "leader," which was included among the criteria used for describing the stage of development of the embryo.

Measurements of the beak and toe, which are the main criteria for identifying chick embryos from stage 40 to 44, were obtained for the turkey embryo. However, owing to the relatively small increments of increase in length in these structures, measurements of the foot, i.e., from the outer edge of the tarsal joint to the tip of the claw of the third toe, were used to characterize the development from seventeenth to the twenty-seventh day of incubation.

This study is based on the observation of more than 4000 embryos collected over a period of three years.
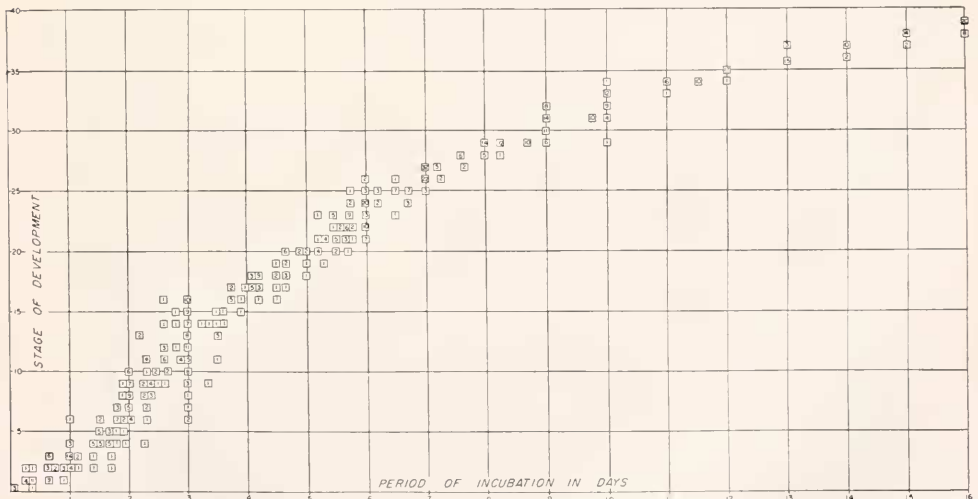

FIGURE 1. Stage (Hamburger and Hamilton, 1951) of development of the turkey embryo after various periods of incubation. The figures in boxes indicate the number of specimens for each point on the coordinate.

TABLE 1

Anteroposterior (AP) lengths of the area pellucida and of the BBB turkey embryo at different stages of development

Stage	No. cases	AP length (mm.)	Sd	Embryo length (mm.)	Sd
4	11	3.3	.375	—	—
5	132	3.9	.423	—	—
6	233	4.3	.400	1.4*	.390
7	101	4.7	.458	2.3*	.242
8	52	5.3	.454	3.1**	.341
9	2	5.6	—	3.8	—
10	5	6.3	—	4.6	—
11	9	7.0	.508	5.6	.225
12	12	7.0	.673	5.8	.310

* From the head fold to Hensen's node.
** From the tip of the head to Hensen's node.

Results and Discussion

The approximate periods of incubation to obtain stages 1 to 39 can be obtained from Figure 1. There is a wide range of variation in the stage of development in the turkey embryo after a definite period of incubation. This becomes particularly apparent in the early stages.

In the earlier stages (stages 4 to 12) the stage of the embryo can also be estimated from measurements of the anteroposterior lengths of the area pellucida or of the embryo, i.e., from the tip of the head, or in stage 6, from the head fold to Hensen's node (Table I). These measurements are highly correlated with the stage and somite number (Mun and Kosin, 1958).

The development of the snood ("leader") is summarized in the following tabulation:

Day of incubation	Hamburger & Hamilton Stage	Snood characteristics
13th day	36	Snood appears (Fig. 2).
12 to 14	37	Snood is as high as it is wide at the base (Fig. 3).
15	38	Snood is higher than wide and distinctly columnar in appearance (Fig. 4).
16 to 17	39	Snood is columnar and almost twice as tall as it is wide. Papillae may be seen at the base of the snood (Fig. 5).
17	40	The snood is covered with papillae (Fig. 6).
18	—	The snood is larger and conical feather germs at base of snood are colored (Fig. 7).
19th day	—	The feather germs are as long as the snood and may cover it completely (Fig. 8).

The growth of the beak, toe, and foot from the seventeenth to the twenty-seventh day of incubation is presented, graphically, in Figure 9. Each point represents the average measurements of 10 to 21 embryos by three different individuals on

FIGURE 2.   Snood at 13th day of incubation.
FIGURE 3.   Snood at 14th day of incubation.
FIGURE 4.   Snood at 15th day of incubation.

FIGURE 5. Snood at 16th day of incubation.
FIGURE 6. Snood at 17th day of incubation.
FIGURE 7. Snood at 18th day of incubation.
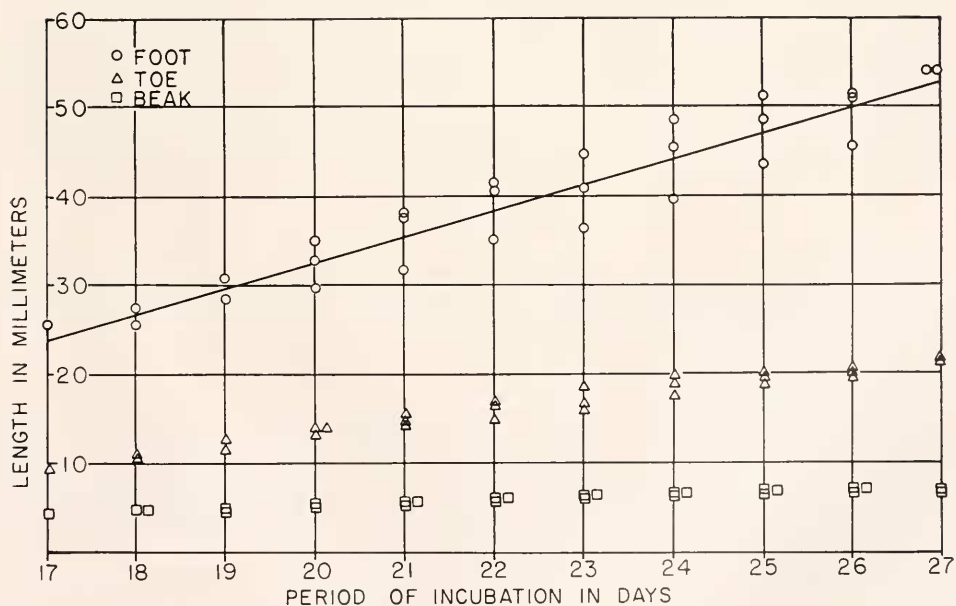
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FIGURE 8. Snood at 19th day of incubation.

FIGURE 9. Length of beak, toe, and foot after different periods of incubation.

different groups of embryos at different times. The measurements were made with a pair of vernier calipers.

As may be seen from Figure 9, the rate of growth of the foot is greater than the rate of growth of the beak and toe. A straight line obtained from the regression of the length of the foot on time was then constructed. The equation of this line (solid line in Figure 9) is as follows:

$$Y = -25.3 + 2.9x$$

where Y = length of the foot in mm. and x = period of incubation in days.

The standard deviation of the regression line is 3.3 and the standard error of the slope is 0.051.

The 95% confidence interval in age of the embryo, for any particular length of the foot, can be calculated from the following equation:

$$\left.\begin{array}{l} \bar{L}x \\ Lx \end{array}\right\} = 36.6 + 0.35Y_0 \pm 0.7\sqrt{0.00024(Y_0 - 40.0)^2 + 8.342}$$

where $\bar{L}x$ = upper limit of age of the embryo in days, Lx = lower limit of age of the embryo in days and Y_0 = observed length of the foot in millimeters.

Similarly, the 95% confidence interval for the length of the foot following a definite interval of incubation can be calculated from the following equation:

$$\left.\begin{array}{l} \bar{L}y \\ Ly \end{array}\right\} = -25.3 + 2.9X_0 \pm 6.47\sqrt{1.002 + 0.00024(X_0 - 22.6)^2}$$

where $\bar{L}y$ = upper limit of the length in mm., Ly = lower limit of the length in mm. and X_0 = observed period of incubation in days.

This information has been used in our laboratory to approximate the time of death of the embryo, whether it was accidental, *e.g.*, due to incubation failures, or due to causes associated with the problem of hatchability, and to compare the growth rates of embryos from different lines of BBB turkeys cultivated *in vitro.*

SUMMARY

1. The period of incubation of Broad Breasted Bronze turkey eggs necessary to obtain the various normal stages of development established by Hamburger and Hamilton for the chick embryo is presented.

2. Data have been submitted on the development of the snood ("leader") in the turkey embryo.

3. Measurements of the beak, toe, and foot were obtained from the seventeenth to the twenty-seventh day of incubation. From these measurements, a straight line obtained from the regression of the length of the foot on time was constructed. The equation of this line is presented, as well as equations for determining the approximate age of the embryo from measurements of the foot, or the approximate length of the foot.

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